

Pathological and molecular studies on tropical theileriosis in a HF cross cow calf

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ABSTRACT

Bovine tropical theileriosis (BTT), a tick borne lymphoproliferative disease of bovines, is caused by intracellular hemoprotozoan, *Theileria annulata*. It is one of the most economically significant disease of livestock in India. The blood sample from a month old HF cross cow calf with the history of high fever, anorexia and weakness was presented for hematological examination to Department of Veterinary Pathology, KNPCVS, Shirwal. The hematological analysis revealed anaemia as evident by decrease in Hb (6.5g/dl) and PCV (20.68%) values. The blood smear examination revealed piroplasms (annular rings/rods) of *Theileria* spp. in RBCs. The calf was died on same day and thorough necropsy was performed to note gross lesions. The carcass showed emaciation, pale/anaemic mucous membranes, swollen edematous prescapular lymphnodes, typical punched out ulcers in abomasum, multifocal epicardial ecchymotic hemorrhages along with paleness in myocardial muscles were salient gross findings. The microscopic examination of impression smear from lymphnode revealed typical Koch blue bodies in lymphocytes. The histopathological examination of affected tissues corroborated with gross findings. DNA was extracted from the blood sample and PCR targeting Tams 1 gene specific for *Theileria annulata* yielded expected product of 751 base pair confirming the tropical theileriosis.

Keywords: HF cross cow calf, Lesions, PCR, Tams 1 gene, *Theileria annulata*

INTRODUCTION

India with its tropical climate has widespread distribution of several vector borne diseases like theileriosis, anaplasmosis, babesiosis, trypanosomosis and ehrlichiosis. Theilerial species infecting bovines can be broadly divided into transforming Theilerial species (*T. annulata* and *T. parva*) and non-transforming species (*T. orientalis*/ *T. sergenti*/*T. buffalei* complex).

Theileria annulata responsible for tropical bovine theileriosis is the most economically important tick transmitted pathogen species¹. Infection prevalence has been reported from several states across the country, including Andhra Pradesh, Telangana, Haryana, Punjab, Gujarat, Uttar Pradesh, Uttarakhand, West Bengal, Chhattisgarh, Maharashtra, Madhya Pradesh, Kerala, Odisha, Karnataka, and Tamil Nadu. Serological surveys indicated 30-60% cross bred cattle positive for *T. annulata* specific antibodies, all over India, except in Himalayan regions. In one of the most recent surveys in Maharashtra has reported 24.5 % cases positive for theileriosis by molecular methods in four different agro climatic zones².

T. orientalis which causes oriental theileriosis has also been reported in India³. In Kerala, examination of blood smears from 246 apparently healthy adult crossbred cattle (Jersey, Holstein Friesian, and Swiss Brown) demonstrated a 26.82% prevalence of *Theileria orientalis* infection⁴. The disease is characterized by carriers status and these animals play role in biomagnification of infection in population. East Coast fever has however not been reported in India due to the absence of its vector⁵.

The disease primarily affects exotic, crossbred cattle and young indigenous calves. The sub-clinical form of the disease has been linked to reduced milk production in cattle, while the severe form leads to high mortality. Tropical

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theileriosis is especially severe in European cattle breeds, with a mortality rate ranging from 40% to 90%. In contrast, the mortality rate in indigenous cattle breeds from endemic regions can be as low as 3%. Numerous fatal clinical cases of bovine tropical theileriosis have been reported worldwide. A tentative diagnosis is made based on clinical signs and confirmatory diagnosis is achieved through microscopic examination of Giemsa-stained blood smears, lymph node fine needle aspirate smears, and the use of serological and molecular

techniques⁶. Although bovine tropical theileriosis caused by *Theileria annulata* is widely reported in adult and crossbred cattle in India, detailed reports describing the clinicopathological alterations and molecular confirmation of fatal infection in very young calves are limited. Therefore, the present study describes the hematological changes, gross and histopathological lesions, along with molecular confirmation of *Theileria annulata* infection in a one-month-old HF cross cow calf.

MATERIALS AND METHODS

The present investigation was conducted on a HF cross cow calf. The blood sample from a month old HF cross cow calf with the history of high fever over 104 °F, anorexia and weakness was presented for hematological examination to the Department of Veterinary Pathology, Krantisinh Nana Patil College of Veterinary Science, Shirwal.

Hematology

The complete blood count was performed by automated hematology analyzer (Abacus Jr. Vet.5, Diatron, Hungary) and blood sample was kept at -20°C for molecular studies. Giemsa stained blood smear was examined for the presence of parasites.

Necropsy and Histopathology

The animal died on the same day of hematological examination and a thorough post-mortem examination was performed to note gross lesions. Tissue samples of affected lymph nodes and other visceral organs were collected in 10% neutral buffered formalin for routine histopathological examination. Impression smear was prepared from affected lymph node and stained with Giemsa staining technique for cytological examination. The formalin fixed tissues were processed by paraffin embedding technique, blocks were prepared, sectioned at 5 µm and stained with hematoxylin and eosin (H&E) for histopathological examination⁷.

DNA extraction & PCR

DNA was extracted from affected blood sample by using phenol chloroform method⁸. Micro tubes containing the extracted DNA were stored at -20 °C until further use. The PCR was prepared in a 50µl reaction mixture, which included 25µl of PCR Master Mix, 3µl of each primer (F:5'-CCGTTAATGCTGCAAATGAGGAGG-3', R: 5'-GAGGCGAAGACTGCAAGGGGAG-3' from Eurofins Genomics India Pvt. Ltd., Bengaluru)⁹ and 5 µl of DNA template. The volume was then adjusted to 50 µl with nuclease-free water. PCR amplification was performed in an automatic thermocycler (Applied biosystems), starting with an initial denaturation step at 95°C for 5 minutes. This was followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 57°C for 1 minute, and elongation at 72°C for 1 minute. A final extension

step was carried out at 72°C for 10 minutes. The amplified products were visualized by electrophoresis in a 1.5% agarose gel stained with ethidium bromide.

RESULTS

Hematological Examination

The hematological examination of blood sample from the HF cross calf affected with theileriosis revealed decreased Hb (6.5g/dl), PCV (20.68%) and total erythrocyte count ($3.6 \times 10^6/\mu\text{l}$) and severely hypochromic RBCs on microscopic examination confirming anaemia. The MCV value was 36.93 fl and MCHC 31.43 gm/dl. The values for total leucocyte and platelet count were $2.75 \times 10^3/\mu\text{l}$ and $260 \times 10^3/\mu\text{l}$ respectively. Thin blood smears were prepared, fixed with absolute methanol and stained with Giemsa stain. The differential leukocytes count revealed 30% neutrophils and 70% lymphocytes. The microscopic examination blood smear showed mostly annular ring shaped piroplasms of *Theileria* spp. in the erythrocytes (Fig. 1). However few rod and ring shaped piroplasms were also evident.

Necropsy and histopathological findings

The calf was died on the same day of confirmation of theileriosis in the laboratory. The necropsy examination revealed pale/anaemic mucous membranes, diffusely and severely swollen edematous & hemorrhagic prescapular lymphnodes (Fig. 2). Impression smear from affected lymphnode stained with Giemsa stain showed presence of typical Koch Blue Bodies in cytoplasm of many lymphocytes (Fig. 3). Enlarged and congested spleen, typical multifocal generalized punched out ulcers in abomasum (Fig. 4), multifocal hemorrhages and ulcers in urinary bladder, mildly pale/anaemic kidneys with focal hemorrhages, multifocal epicardial ecchymotic hemorrhages along with paleness in myocardial muscles (Fig. 5), diffusely edematous lungs and swollen pale liver with flat whitish foci as salient gross findings. Histopathologically, lymph node revealed lymphocytic proliferation and degenerative changes in the germinal centre along with infiltration of plasma cells and macrophages in cortex and paracortex region (Fig. 6). Heart muscle showed degeneration of cardiac myofibers along with mild infiltration of lymphocytes between muscle fibres (Fig. 7). Multifocal areas of mild tubular degeneration and necrosis, focal mild hemorrhages along with mild interstitial lymphocytic infiltration were evident in both the kidneys. The lung parenchyma revealed presence of pinkish edematous fluid in alveolar spaces along with congestion of alveolar capillaries and focal hemorrhages. The histopathological changes in other organs corroborated with gross lesions.

Molecular confirmation

For molecular confirmation of involvement of *T. annulata*, the PCR targeting *Tams 1* gene specific for

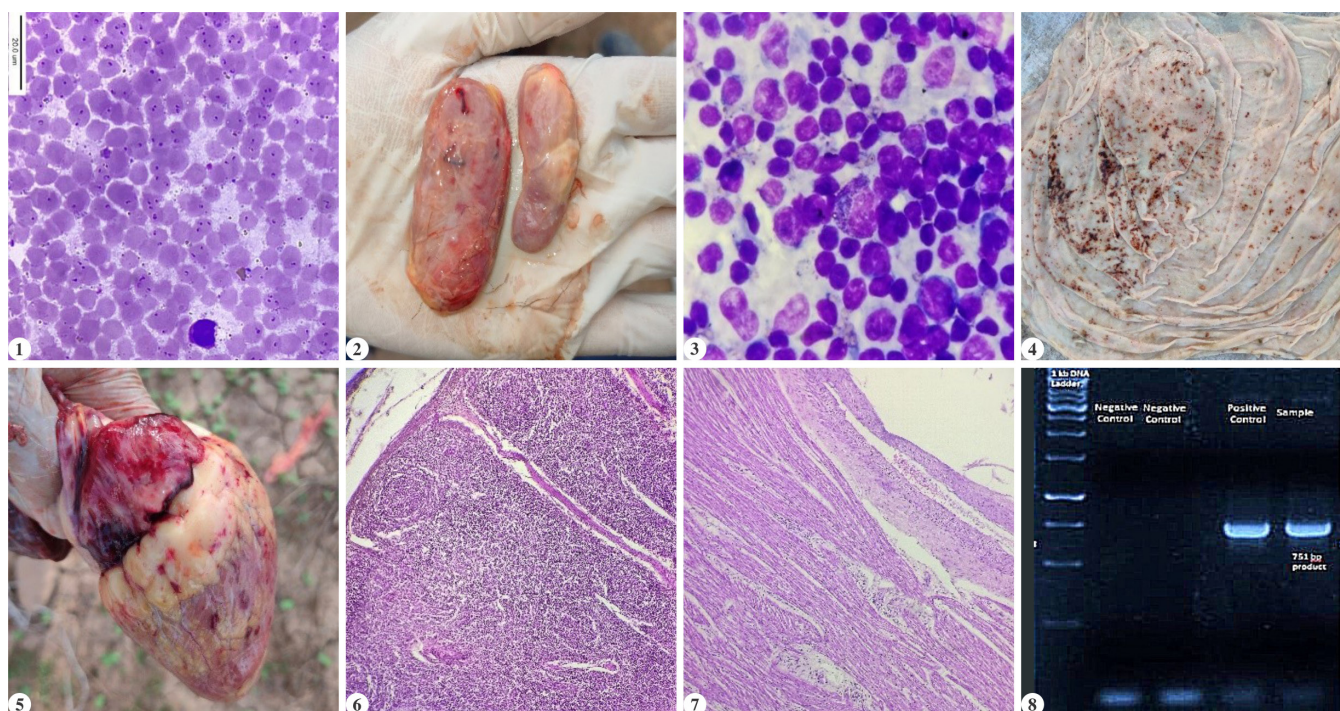


Fig. 1. Giemsa stained thin blood film showing intra-erythrocytic annular ring shaped piroplasms of *Theileria* spp. x1000; **Fig. 2.** Enlarged, edematous prescapular lymphnodes; **Fig. 3.** Lymphnode impression showing Koch's blue bodies in lymphocytes. x1000; **Fig. 4.** Typical punched out ulcers in abomasum; **Fig. 5.** Multifocal epicardial ecchymotic hemorrhages along with paleness in myocardial muscles; **Fig. 6.** Lymphnode showing lymphoid hyperplasia in cortex and pericortex region. H&E x100; **Fig. 7.** Degeneration of cardiac myofibers along with mild infiltration of lymphocytes. H&E x100; **Fig. 8.** PCR targeting Tams 1 gene specific for *Theileria annulata* showing positive amplification to yield product of 751bp.

Theileria annulata was performed on the DNA sample extracted from blood sample of affected animal. The PCR yielded expected product of 751 base pair (Fig. 8) confirming the tropical theileriosis.

DISCUSSION

Seven species of *Theileria* are known to infect cattle, of these *T. parva* and *T. annulata* are considered the most pathogenic and economically significant. The other five species are less pathogenic, and some of them may confuse the epidemiology of theileriosis. Various criteria and methods have been used to identify these parasites. Identification of *Theileria* species based solely on morphological characteristics of piroplasms and schizonts in blood or lymphoid tissues can be challenging, as these stages often show considerable overlap among species. Moreover, mixed infections with different *Theileria* species may further complicate microscopic identification. Therefore, molecular diagnostic methods are considered more reliable for accurate species confirmation.

The anemia observed in the present case may be attributed to multiple pathogenic mechanisms associated with *Theileria annulata* infection. The parasite invades and multiplies within erythrocytes, leading to direct damage and destruction of infected red blood cells. In

addition, immune-mediated erythrophagocytosis by macrophages contributes to the removal of parasitized erythrocytes from circulation. The reticuloendothelial system, particularly in the spleen and liver, actively clears infected erythrocytes, further reducing the circulating red cell population. Activation of complement pathways and increased oxidative stress may also promote erythrocyte membrane damage and hemolysis, thereby exacerbating the anemia observed in infected animals¹⁰.

The major gross lesions observed during necropsy included enlargement, edema, and hemorrhages in the lymph nodes and spleen, punched-out ulcers in the abomasal mucosa, and multifocal ecchymotic hemorrhages on the epicardium. These lesions are consistent with the systemic nature of tropical theileriosis. Histopathologically, the lymph nodes and spleen showed marked proliferation of lymphoid cells within the hematopoietic regions, reflecting intense stimulation of the lymphoid system. This change occurs because *Theileria annulata* primarily infects and transforms lymphoid cells, leading to uncontrolled proliferation of infected leukocytes and resulting lymphoid hyperplasia. In addition to lymphoid tissues, infiltration of lymphocytes and macrophages was observed in the intermyoseal spaces of the heart and in the interstitial tissue of the kidneys. These inflammatory changes may result from

the systemic dissemination of infected leukocytes and the host immune response against parasitized cells. Increased activity of reticular cells in lymphoid organs has also been suggested to represent a protective response aimed at limiting the spread of infection. Similar pathological alterations involving the spleen, epicardium, myocardium, and kidneys have been documented in earlier studies¹¹ of tropical theileriosis, supporting the observations recorded in the present case.

PCR targeting the *Tams-1* gene specific for *Theileria annulata* yielded an expected amplicon of 751 bp, confirming the presence of tropical theileriosis in the affected calf. The *Tams-1* gene encodes an immunodominant merozoite surface protein and is widely used as a reliable molecular marker for the detection of *Theileria annulata*. Similar molecular confirmation based on the *Tams-1* gene has been reported by previous studies¹².

Theileriosis is a economically important disease. There were reports on theileriosis with blood smear examination and molecular studies, but the reports on necropsy findings in young calves were sparse in this region, hence the findings of current study adds to the knowledge on clinical findings, gross and microscopic lesions and etiology of bovine tropical theileriosis.

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REFERENCES

1. Bhatnagar CS, Bhardawaj B, Sharma DK & Meena SK. 2015. Incidence of haemoprotozoan diseases in cattle in Southern Rajasthan, India. *Int. J. Curr. Microbiol. App. Sci.* **4(3)**: 509-514.
2. Kolte SW, Larcombe SD, Jadhao SG, Magar SP, Warthi G, Kurkure NV & Shiels BR. 2017. PCR diagnosis of tick-borne pathogens in Maharashtra state, India indicates fitness cost associated with carrier infections is greater for crossbreed than native cattle breeds. *Plos one* **12(3)**: e0174595.
3. Brahma J, Baishya BC, Phukan A, Mahato G, Deka DK & Goswami S. 2018. Prevalence of *Theileria orientalis* in cross-bred cattle of Kamrup district of Assam. *International Journal of Chemical Studie* **6**: 1791-94.
4. Kariyappa PR, Ravindran R, Nimisha M, Amrutha BM, Kurbet PS, Kumar KGA & Dinesh CN. 2017. Prevalence of bovine babesiosis and theileriosis in Kerala, India. *Int. J. Curr. Microbiol. Appl. Sci.* **6(8)**: 2310-2314.
5. Singla LD & Kaur P. 2022. A comprehensive review on bovine tropical theileriosis under Indian scenario. *Indian J. Vet. Med. Vol.* **42(2)**: 1-12.
6. Azhahianambi P, Madhanmohan M, Madan N, Kumaran D, Priyadharshini MLM, Bharathi R & Manoharan S. 2021. Successful treatment of severe form of bovine tropical theileriosis in dairy cattle and genotyping of *Theileria annulata* isolates of Tamil Nadu, India. *Veterinary Parasitology: Regional Studies and Reports* **26**: 100628.
7. Luna LG. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd Edition, McGraw-Hill, New York.
8. Sambrook J, Fritsch EF and Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Press, New York.
9. Kundave VR, Nehra AK, Ram H, Kumari A, Shahzad M, Vinay TS & Tiwari AK. 2021. Genetic diversity in the *Tams 1* gene of *Theileria annulata* (Duschunkowsky and Luhs, 1904) infecting cattle. *Acta tropica* **224**: 106121.
10. Agrawal V, Das G, Singla LD, Shukla S, Maharana BR, Jayraw AK & Jatav GP. 2023. Bovine cerebral theileriosis: first molecular report in cross bred cattle calf in India. *Journal of Parasitic Diseases* **47(1)**: 113-117.
11. Gill BS, Bhattacharyulu Y & Kaur D. 1977. Symptoms and pathology of experimental bovine tropical theileriosis (*Theileria annulata* infection). *Annales de parasitologie humaine et compare.* **52(6)**: 597-608.
12. Rajendran C, Ray DD & Bansal GC. 2008. Expression of gene encoding immunodominant merozoite surface protein of *Theileria annulata* in *Escherichia coli*. *Indian J Biotechnol* **7**: 200-203.